August 20. The temperature of the water was tested at another location in the same lake. The test was made at 5 a.m. near the shore in water half an aln deep. The thermometer registered 14° in the air but 18° in the lake. At 5:30 a.m. the temperature of the air was 14° and that of the lake water 18° at a rifle-shot distance from shore and a depth of a famn.

On a hillside between Burnets field and Albany there was a spring whose water seemed unusually cold for drinking. It was tested on September 8 at 11:30 a.m. in the year just mentioned. Part of the spring was exposed to the sun, and part of it lay in the shade. The temperature of the open air was 22°, but that of the spring was 6° as shown by several tests. To find water registering only 6° was most remarkable because the temperature of the air had for a long time prior to these tests ranged from 22° to 31° This was the coldest spring water I found in America.

1751, January 17. I again tested the temperature of the water of the same deep well in Philadelphia which I had tested on May 19 and July 4 of the previous year. At 7:30 a.m. on January 17, the temperature of the air was 7° below the freezing point, but the temperature of the water pumped from the well registered 11° to 11½° above the freezing point. The thermometer remained in the bucket during the entire time that the water was being pumped. The pumping continued until the water flooded the board covering of the well. The temperature for the entire period varied from 11° to 11½° above the freezing point. It is obvious from the foregoing that the well water in Philadelphia has nearly the same temperature for both summer and winter. However, it should be noted that the wells were very deep, and those that I tested had pumps. The wells were covered, preventing both daylight or sunlight from reaching into them.

January 28. At 7 a.m. in Philadelphia the temperature of the air was 4° above the freezing point. The temperature of the water in the Delaware River, which was full of floating ice. was tested several times, and it remained at ½° above the freezing point. At 2 p.m. the temperature of the air was 9° above the freezing point. However, the temperature of the water in the river, which still contained floating ice, remained the same as in the morning, ½° above freezing.

BIOLOGY.—*Fungus-growing ants and their fungi:* Cyphomyrmex rimosus minutus *Mayr*. Neal A. Weber, Swarthmore College, Swarthmore, Pa. (Communicated by F. L. Campbell.)

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The most widespread fungus-growing ant, *Cyphomyrmex rimosus minutus* Mayr, is also one of the smallest and least conspicuous. It is the only fungus-grower generally distributed on the West Indian islands, and the species occurs with subspecific differences from the Gulf States of the United States south to Bolivia and Brazil. Wheeler (1907) described the habits of the species (as the variety *comalensis*) in Texas and its fungus as *Tyridiomyces formicarum*, a yeast belonging to the Exoaceae. The general biology in several countries has been described in a series of studies (Weber, 1941–1947). The morel-like growth on sterile nutrient agar from a pure culture of the ant's fungus was obtained in 1935 and again in 1954 (Weber, 1955). No other species is known to grow a fungus resembling this.

In the present study hitherto unknown relations with another ant and with mites are described and information given on the culture of the fungus. The field studies in south-central Florida were made possible through the assistance of Richard Archbold and the Archbold Biological Station; additional material was secured by Leonard Brass here.

HABITAT

The ant is versatile in the American Tropics and may be found in a great variety of sites where the humidity is high and temperatures uniform. The most common sites are in clay soil on the forest floor, in humus, or in vegetal debris comprising the floor litter. An empty snail shell, a curled dead leaf, a rotted twig may suffice for a colony of these small ants and they may find requisite conditions among the roots of epiphytes or in dead wood high in the tropical rain forest canopy. Reflecting their versatility in Panamá City, Panamá, during the rainy season in July 1954 was a nest on the concrete cylinder above ground which protected a gas meter. The cylinder was 17 cm high by 36 cm in diameter and was covered loosely by a concrete cover. In the narrow space on the rim under the cover, a colony had walled off an elliptical area 36 by 17 mm and 1–2 mm high in which the entire nest with fungus-garden was formed. During dryer periods the ants would move down into the soil.

The ants show less versatility in southern Florida where the humidity and temperatures are more variable. At Key West the ants nested in porous limestone soil whose surface layer would quickly dry out. Winged males and females August 18 were clinging under a dry, flat rock while the brood and garden were kept in much deeper chambers. At Parker Islands (Highland County) the ants nested at the base of a large cabbage palm (*Sabal palmetto* (Walt.)) in a swampy area. In Highland Hammock State Park the ants nested at the base of a pine tree (*Pinus elliotti* Engelm.) in the midst of a large colony of *Wasmannia auropunctata* Roger. Both species nested between the paper-thin layers of bark at and just below soil level. During the rainy season at the Archbold Biological Station in August 1954 the ants were found nesting under wood in soil chambers, in soil about fern roots at the edge of a pool and in a rotted stump of wax myrtle (*Cerothamnus ceriferus* (L.)), all in shaded sites as in the Tropics. The rains were too intermittent to permit more widespread nesting.

THE NEST

The Florida nests were representative of those in the Tropics. The ants form irregular chambers when they do not occupy a cavity already made and these are a few millimeters in dimensions. The fungus-garden consists of the small, compact masses or bromatia stuck to the substrate. Insect excrement is commonly used and frequently the bromatia rest on pieces of insect integument that the ants bring in to the nest. The brood is kept separate from the garden in contrast to all other species, which keep the brood in the cells of the garden.

One of the Archbold Station nests was at the edge of an artificially-maintained pool among fern roots and humus in the shade. Since the roots and humus extended to water line, the site was always moist. The ants had irregular chambers in the top 2–5 cm of material and were in the midst of a large nest of *Wasmannia auropunctata* Roger. Thirty meters distant from this nest was one in the above-noted wax myrtle stump, that itself was in the midst of a thicket of palmetto and other plants. In August the ants nested slightly above and below the general ground level. This colony, too, was in the midst of a far larger *Wasmannia* nest.

ADAPTATIONS TO THE FLORIDA WINTER

Of the two fungus-growers at the Archbold Station, this species and *Trachymyrmex septentrionalis seminole* Wheeler, the latter is far more tolerant of winter coolness and dryness. During a few days visit in December 1954 the coldest weather of the winter occurred and permitted observations on winter tolerance.

At the pool margin where an active nest had existed in August, the pool water was 14° C. at 7 a.m. on December 21; lawn grass in the open was covered with frost at 0° C. The humus surface at the pool was 5.6° C. and at 100 cm in the air it was 5.2° C. These were the lowest temperatures in the December 20–26 period and daily search of the area, including superficial layers of roots and humus at the exact summer nest site, showed the *Cyphomyrmex* to be absent until the 26th. By this time the weather had gradually warmed so that by 2:10 p.m. the humus surface temperature in the shade was 22.5° C. (in the sun 24.7° C.) and the shade air temperature at 100 cm was 23.7° C. At a depth of 2.5 cm in the humus at 2:53 p.m. the temperature was 18.5° C. and at 5 cm 18.0° C. Several workers were carrying others and this would be a habit permitting a few, which had become warmed, to carry out others, thereby making possible a more general activity of the colony at the temperature threshold. The ants went down irregular openings among the roots close to a large orange spot consisting of solidly packed *Wasmannia* workers under a dry leaf, which were also becoming warmed at the surface. One *Cyphomyrmex* worker carried a larva that was covered with the characteristic fungus of the species. It seemed that a few of the ants were moving from one site near the pool water to a more distant site.

The waxmyrtle stump appeared to contain only the large *Wasmannia* colony on December 20–26. The ants were sluggish during the cooler weather and still more adaptable than the *Cyphomyrmex* in tolerating dryer conditions of the rotted wood. None of the fungus-growers was found in deeper and adequately moist portions.

It would appear that about 18° C. is the critical lower limit of *Cyphomyrmex* activity and that even at 22° C. there is little moving about. Close to the Gulf coast at Fort Myers on December 23 several workers were found at the same shaded place as in August and the soil surface temperatures here were more generally in the 20°'s C.

BEHAVIOR OF THE ANTS

Though the workers are usually slow-moving and become immobile at the slightest disturbance, they frequently moved faster in Florida during the summer than at the usual tropical site because of the higher temperatures. The air temperature was often 30° C., to a high of about 37° C., and the soil surface also markedly warmer (30° C. and more) than they generally encountered in the tropics. Instead of moving slowly, they would, when pursued, sometimes run as rapidly as the average ant and sought to escape rather than "feign death." In "feigning death," the ants quickly curl up their legs, fold their antennae close to the head and bend their heads and gasters together so that they appear almost invisible bits of dirt when casually examined.

The ants spend much time in grooming the forelimbs and antennae and other parts of the body. Regardless of how dusty an ant may become momentarily, it keeps its antennal funiculus immaculate by drawing it through its mouthparts, with mandibles widespread, and licking and cleaning it. The comb at the base of the foretibiae is used to clean all parts of the body within reach, particularly the antennae and other legs. They also clean one another. In grooming each other the ant may carefully go over a large portion of the body. In one instance a slightly callow worker was watched as it groomed another of the same age. The one being groomed turned over on its side, like a dog or monkey would, and permitted the other to lick the entire gular surface thoroughly, the entire dorsal surfaces of the thorax and gaster, the apex of the gaster and other parts for over three minutes. The groomer kept its mandibles closed, extruding the other mouthparts, and continually played its antennal tips against the parts being cleaned. The grooming of each other and the cleaning of the brood is a significant and vital part of their activities. It removes alien bacteria and fungi and may also have a nutritive function so far as the brood is concerned.

Though the normal food of the ants consists of their bromatia, they will feed on their own damaged brood. A larva that was accidentally damaged in collecting the colony was seen to be pinched hard by the mandibles of two workers and its juices were lapped up. The mandibles could be seen meeting through the integument. Four ants were later on it, feeding intently. The integument is sufficiently tough and turgid in the healthy large larvae and pupae so that the ants would have considerable difficulty in piercing it. On another occasion the carcass of a shrivelled white pupa was seen carried that had been treated in the same manner.

The behavior of the ants with their bromatia is described under the fungus gardens.

CARE OF BROOD

The brood is kept separate from the garden and is segregated according to size; large larvae may be mingled with pupae. The brood is usually enveloped in a mycelium that differs from that in other attines in being almost granular in superficial appearance, consisting of dense masses or tufts that are always connected by ordinary hyphal strands. Under a 32× binocular the tufts show as a more concentrated form of bromatia than in other attine species. This type of mycelium with hyphae differs markedly from the cheese-like bromatia found in the garden of these ants. About 200 tufts were estimated to be on one pupa. Eggs and the smallest larvae as well as larger brood may be covered with the mycelium. The position and frequency of the tufts indicate that they sometimes may be planted by the workers.

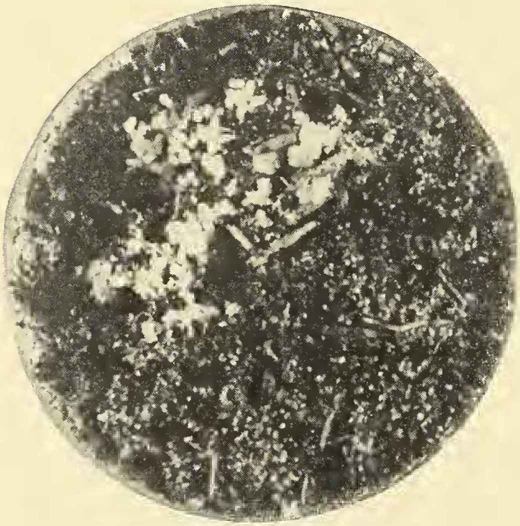

Fig. 1.—Nest of *Cyphomyrmex rimosus minutus* Mayr in a Petri dish. The brood and fungus-garden are segregated, larvae at the left, pupae at the upper center, and bromatia of the garden at the right. The brood is covered with a mycelium of different form from the bromatia but developed from them.

Larvae are fed as in other attines by placing the fungus on the mouthparts. In this species the fungus fed to the larvae seems to consist only of the cheeselike bromatia. As the larva feeds, the mouthparts go in and out like pistons while the bromatium is rasped and the juices imbibed.

CYPHOMYRMEX FUNGUS-GARDENS

The fungus-gardens consist of polygonal masses one-quarter to one-half millimeter in diameter that are termed bromatia although the bromatia of all other species are very different. Instead of aggregates of clavate hyphae (gongylidia) clustered together in varying degrees of compactness, the bromatia of *Cyphomyrmex rimosus* are solid masses of cells. They usually grow on insect feces, but carcasses of insects are commonly found in the nest with bromatia stuck to them. In feeding, an ant will pick up a bromatium, hold it between its fore feet and mandibles, rotate it with the fore tarsi while the mouthparts abrade the surface or score it, and imbibe the juices. If the mandibles are kept closed, the other mouthparts do the abrading; if they are kept open, they are used to score the surface of the bromatium. The antennal tips play over the bromatial surface continually. One ant may take a bromatium from another and commence eating without evoking hostility. During this treatment the bromatium becomes much reduced in size and glistening from its juices and the saliva of the ant. It is then placed back on the substrate, sometimes after defecating a drop of feces on it.

At times a score or more of the bromatia may be piled together in the nest away from substrate. Small bromatia may be somewhat of an opaque, dead white; larger ones are commonly pale amber or grayish with a touch of brown. In a pile the bromatia may develop a short, scanty growth of hyphae and this is particularly true of the small, pale type. This hyphal development is apparently intermediate between the bare bromatia and the tufted mycelial covering of the brood.

ARTIFICIAL FUNGUS CULTURES

Bromatia were transferred to tubes of sterile Sabouraud's dextrose agar and commonly contaminations developed which overwhelmed the ant fungus. An August 5, 1954, series of transfers, for example, developed by August 9 (at 24° C.) concentric layers of dark and light hyphae about a white center which produced an "eyed" effect; another contaminant produced a luxuriant cottony mycelium; a third developed a bacterial or yeasty slime about the bromatia. From a single bromatium transferred August 11, however, an entirely different growth developed. It was kept until August 22 at 24° C. and thereafter under variable temperatures. On August 12 it appeared to have grown, and on August 14 it had a few thin hyphae growing out on the agar surface from a definitely larger mass. By August 17 it had not only increased in size but had developed a raised base that was covered with hyphae. By August 22 the much larger mass was transferred to a flask. In the meanwhile, an August 11 transfer of a cluster of about a score of bromatia, kept under the same conditions, by August 20 had increased in number through budding and the individuals had also grown larger. By August 21 this mass had increased in height from an original 1–2 mm to 5 mm on a broad base 1 mm. thick.

Both cultures and their transfers developed during September a morel-type of fungus covered with a thin growth of short hyphae that closely resembled that which I grew in 1935 in Trinidad (Weber, 1945) from the same species of ant. This was verified as the true ant fungus as follows:

On September 13 the flat basal layer of one of the cultures was cut into two pieces and introduced into separate containers, each containing ants from a colony different from that which served as the original source for the artificial cultures. The layer was tough in consistency. These ants had been deprived of their fungus since approximately August 26 but had access to corn sugar syrup in the interval. At first the ants only occasionally investigated the cultured fungus and seemed to feed briefly, but it was not clear whether they were feeding on a film of agar or on the fungus. Five hours later it was clear that the fungus was the attraction and seven, then eight, ants were in attendance in one container, all busily engaged in cutting or attempting to cut the tough piece. This fungus had the same color and consistency as their normal bromatia. Two ants were similarly engaged with the second portion. Within two more hours the ants had cut small pieces about the size of their normal bromatia, or smaller, and had piled them in a moist situation. On following days the ants cared for these pieces as though they were their own bromatia and by September 22 consumed all in one of the containers. The experiment was then repeated with this group of ants and a third fungal mass, with similar results. The morel growth proved to be 0.43–0.49 mm thick, whether folded or lying flat. Within three hours the ants had cut much of the fungus into bromatia-size particles. Five days later the remainder and uncut fungal mass was sprouting hyphae wildly and was clearly abandoned, while the artificial bromatia were being cared for and carried about normally.

Fig. 2.—Morel-like growth from bromatia of *Cyphomyrmex rimosus minutus* Mayr developing in a flask of Sabouraud's agar. Phot. Lloyd Merritts.

During October various portions of colonies were similarly given the artificially cultured fungus with the same result. They were also given feces of wood-boring beetles which they accepted and used as substrate for the bromatia. The ants were often seen clearly to ingest the fungus as in nature. The ants were able to keep these bromatia or their successors alive for more than six months.

RELATIONS WITH WASMANNIA AUROPUNCTATA ROGER

The ranges of *Cyphomyrmex rimosus minutus* Mayr and the much smaller *Wasmannia auropunctata* Roger are comparable in the American Tropics, and both are common ants on many of the West Indian islands. The only previous record of the two being more than casually related appears to be from Venezuela (Weber, 1947, p. 145) where I found that "associated with the *Cyphomyrmex* (*C. rimosus curiopensis* Weber) was a nest of the tiny myrmicine ant, *Wasmannia auropunctata* Roger, whose chambers must have anastomosed with those of the other ant."

Of seven nests of *Cyphomyrmex* examined in southern Florida, three were intimately associated with more populous *Wasmannia* nests and two had the latter nesting in the immediate vicinity. One of the remaining two had recently been formed under a temporarily wet piece of wood and the other nested at the base of a palm in a swampy situation where *Wasmannia* was not taken. *Wasmannia* occurred at two of the sites in December as they did in August and showed a greater tolerance to dry conditions, and perhaps cold, than did the fungus-grower.

When portions of any of these nests were gathered with adjacent *Wasmannia* chambers and placed in observation nests, there was no mass slaughter of either. Rather there was a milling about, with few of the two species meeting in combat, and a rapid reassortment of brood and ants with the two taking up separate places. Nevertheless, with the passage of time, dead ants of both species were commonly found with enough observations to suggest strongly that combats between the two were responsible. Occasionally a *Cyphomyrmex* would be seen with a dead *Wasmannia* attached to an appendage. One, for example, had the smaller ant attached to the left posterior leg and walked easily, considering the impediment. Some of the larger ants were found with all appendages intact and lying on their backs or sides. The observation below shows the effectiveness of the sting of the smaller species.

A *Wasmannia* worker that was not one-third the bulk of the *Cyphomyrmex* found a partly shriveled larva attached to the surrounding soil by a strand of tissue. It fed by licking abrasively and did not attempt to carry the larva away. Immediately one, then two other *Cyphomyrmex* came up to it, stalking like miniature rhinoceroses and walking directly up to the smaller ant. It ignored them, though hostility was indicated by their stance, and kept on feeding. A fourth *Cyphomyrmex* approached the tableau but went off. One of the larger ants finally pushed its head against the *Wasmannia*, which kept on feeding. I then saw that this ant had the common *Cyphomyrmex* type of mite attached to its gular surface and this, too, was extending its palps to the food.

The *Cyphomyrmex* with head next to the smaller ant suddenly attempted to sieze the food, whereupon the *Wasmannia* quickly curled its gaster forward and stung the other ant in the mouth. It recoiled on its back as though momentarily paralyzed, then regained its feet and went off. Close to this scene was another of the larger ants on its back, feebly waving its appendages as though paralyzed and unable to regain its feet. Other *Cyphomyrmex* in this and other nests were in similar attitudes and it is probable that these also had been stung by *Wasmannia*. Ten minutes later the *Wasmannia* was still feeding.

MITES WITH THE CYPHOMYRMEX

The common occurrence of mites with this fungus-grower in Florida is a complication that seems never to have been realized. It has not been observed to a comparable extent in any of the numerous species that I have kept under observation in Tropical America nor is it so recorded in the literature. Eidmann (1937, pp. 403–404) lists mites from *Atta sexdens* nests in Brazil but mostly as coprophiles or neutral synokoetes and never as ectoparasites or in phoresy.

All colonies taken at the Archbold Biological Station contained mites that were riding on the worker ants. Since the ants are small and the mites much smaller they were commonly recognized under the binocular microscope. In this restricted field of vision as many as seven out of sixteen ants in view at once had mites on them although commonly two or three ants out of ten might show mites. Since the mites can quickly leave their hosts and some may be hidden on underparts of the ants a complete census cannot readily be made. Frequently an ant will have more than one mite. Two mites may occupy opposite sides of the ant thorax, may face each other here or be in tandem position. One ant had two on the thorax and one on the underside of the gaster. The head and gaster are also common sites. The mites have no difficulty in moving from one site to another on an ant and hold on tightly with body appressed and mouthparts porrect.

These mites, unfortunately not yet identified, closely resemble those taken on *Trachymyrmex septentrionalis seminole* nesting nearby and may well be the same. Two collections from the latter ant were identified as *Garmania* sp. (Phytoseiidae) by G. W. Wharton.

A transfer of a mite from one ant to another was watched under the binocular. It had been riding on the gaster of one ant when another brushed by, waving its antennae over the other ant as is customary. In a flash the mite grabbed the left antennal tip, taking a position with its head facing proximally, and held tightly. The ant did not attempt to dislodge the mite and had already two others, one on the thorax, the other on the gaster. The mite on the antenna grasped firmly with all legs and kept its palpi appressed as the ant attempted to force its way through a narrow passageway, antennae probing the meanwhile. The mite had a rough ride but was not dislodged.

A mite was watched for more than ten minutes as it fed on a bromatium. It fed from below only, "pecking" at the fungus repeatedly and clearly ingesting it. The palps played continually over it. Some mites had their short mouthparts, in addition to the lateral palpi, appressed to the integument of the ants and may have been ingesting the epidermal secretions.

ANTIBIOTIC AND GROWTH-PROMOTING ASPECTS OF THE SYMBIOSIS

This ant fungus has not been recognized outside of the nest and appears easily to be overwhelmed by other fungi in artificial culture. It is clearly maintained by the activities of the ant and is cultivated only in the form of small compact masses or bromatia. The same fungus grows regularly on the eggs, larvae and pupae in a hyphal form. In feeding on a bromatium, the ant adds its saliva to the fungus and may defecate on it before replacing it in the garden. In licking the brood, a regular feature of attine ant behavior, saliva must also be added to the integument. This may be nutritive for the mycelium, or the mycelium may digest substances from the integument. The ants keep each other immaculate by constant grooming, in which the integument is well licked. The saliva added to the integument may prevent the development of alien fungi and bacteria.

From the above review of behavioral features it would appear that distinctive antibiotic and growth-promoting features are produced in the symbiosis. A study of the roles of the ant feces and saliva is indicated.

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